

In re Application of:  
Knapp et al.  
Application No.: 09/759,629  
Filed: January 12, 2001  
Page 2

PATENT  
Attorney Docket No.: AURO1290-4

## I. AMENDMENTS

### In the Claims

Please amend the claims to read as follows:

~~1.~~ <sup>80.</sup> (previously amended) A system for fluorescence assays, comprising:

- a) a source of excitation light, for fluorescent excitation;
- b) a detector, for measuring emission; and
- c) an aqueous reagent mixture, comprising:
  - i) a plurality of living cells in contact with a solid surface,
  - ii) a first reagent, comprising a photon producing agent,
  - iii) a second reagent comprising a photon reducing agent,

wherein said photon reducing agent is substantially impermeant to a plasma membrane of a living mammalian cell,

wherein said photon reducing agent does not specifically bind to said membrane compartment,

wherein said photon reducing agent has an absorption spectrum that overlaps with the absorption, emission or excitation spectrum of said photon producing agent, and

wherein said photon reducing agent is present in said aqueous reagent mixture at an amount sufficient to reduce light emitted from said aqueous reagent mixture by at least 10 % compared to the light emitted from said aqueous reagent mixture in the absence of said photon reducing agent.

<sup>2.</sup>  
~~81.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said solid surface is a bottom of a multiwell plate.

In re Application of:

Knapp et al.

Application No.: 09/759,629

Filed: January 12, 2001

Page 3

PATENT

Attorney Docket No.: AURO1290-4

~~3.~~  
~~82.~~ (previously added) The system of claim ~~81~~<sup>2</sup>, wherein said multiwell plate has between 6 and 3,456 wells.

~~4.~~  
~~83.~~ (previously added) The system of claim ~~81~~<sup>2</sup>, wherein said multiwell plate has greater than 384 wells.

~~5.~~  
~~84.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, further comprising a temperature controller.

~~6.~~  
~~85.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, further comprising a multi-axis translation stage to move a multiwell plate.

~~7.~~  
~~86.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, further comprising auto focusing optics.

~~8.~~  
~~87.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, further comprising programmable control of imaging and data collection.

~~9.~~  
~~88.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said plurality of living cells comprises a target receptor.

~~10.~~  
~~89.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said plurality of living cells comprises a target ion channel.

~~11.~~  
~~90.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said plurality of living cells comprises a target intracellular nuclear receptor.

~~12.~~  
~~91.~~ (previously amended) The system of claim ~~80~~<sup>1</sup>, wherein said photon reducing agent is present in said aqueous reagent mixture at an amount sufficient to reduce light emitted

52

C

from said aqueous reagent mixture by at least 30 % compared to the light emitted from said aqueous reagent mixture in the absence of said photon reducing agent.

<sup>13</sup>/<sub>92</sub> (previously amended) The system of claim <sup>1</sup>/~~80~~, wherein said photon reducing agent is present in said aqueous reagent mixture at an amount sufficient to reduce light emitted from said aqueous reagent mixture by at least 50 % compared to the light emitted from said aqueous reagent mixture in the absence of said photon reducing agent.

<sup>14</sup>/<sub>93</sub> (previously amended) The system of claim <sup>1</sup>/~~80~~, wherein said photon reducing agent is present in said aqueous reagent mixture at an amount sufficient to reduce light emitted from said aqueous reagent mixture by between 70 and 99 % compared to the light emitted from said aqueous reagent mixture in the absence of said photon reducing agent.

<sup>15</sup>/<sub>94</sub> (previously amended) The system of claim <sup>1</sup>/~~80~~, wherein said photon producing agent is selected from the group consisting of a fluorescent enzymatic substrate, a fluorogenic enzymatic substrate, a member of a fluorescence resonance energy transfer (FRET) pair, a molecule that detects voltage across a membrane of a membrane compartment and an intracellular ion indicator.

<sup>16</sup>/<sub>95</sub> (previously added) The system of claim <sup>15</sup>/~~94~~, wherein said photon producing agent is a fluorescent enzymatic substrate.

<sup>17</sup>/<sub>96</sub> (previously added) The system of claim <sup>15</sup>/~~94~~, wherein said photon producing agent is a fluorogenic enzymatic substrate.

<sup>18</sup>/<sub>97</sub> (previously added) The system of claim <sup>15</sup>/~~94~~, wherein said photon producing agent is a member of a FRET pair.

In re Application of:  
Knapp et al.  
Application No.: 09/759,629  
Filed: January 12, 2001  
Page 5

PATENT  
Attorney Docket No.: AURO1290-4

~~14.~~  
~~98.~~ (previously added) The system of claim ~~94~~<sup>15</sup>, wherein said photon producing agent is a molecule that detects voltage across a membrane.

~~20.~~  
~~99.~~ (previously added) The system of claim ~~94~~<sup>15</sup>, wherein said photon producing agent is an intracellular ion indicator.

~~21.~~  
~~100.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said photon reducing agent is selected from the group consisting of a collisional quencher, a particulate, an absorption quencher, a FRET quencher and a dark complex.

~~22.~~  
~~101.~~ (previously added) The system of claim ~~100~~<sup>21</sup>, wherein said photon reducing agent comprises a particulate or colloidal quencher.

~~24.~~  
~~102.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said photon reducing agent comprises a light absorbing dye.

~~25.~~  
~~103.~~ (previously added) The system of claim ~~102~~<sup>22</sup>, wherein said photon reducing dye is not a pH indicator dye.

~~23.~~  
~~104.~~ (previously added) The system of claim ~~100~~<sup>21</sup>, wherein said photon reducing agent comprises a FRET quencher.

~~26.~~  
~~105.~~ (previously added) The system of claim ~~80~~<sup>1</sup>, wherein said second reagent comprises at least two photon reducing agents.

In re Application of:  
Knapp et al.  
Application No.: 09/759,629  
Filed: January 12, 2001  
Page 6

PATENT  
Attorney Docket No.: AURO1290-4

<sup>27.</sup>  
~~106.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises Tartrazine.

<sup>28.</sup>  
~~107.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises chromotrope 2R.

<sup>29.</sup>  
~~108.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises Acid Fuchsin.

<sup>30.</sup>  
~~109.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises Patent Blue.

<sup>31.</sup>  
~~110.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises Acid Red 37.

<sup>32.</sup>  
~~111.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises chromotrope F8.

<sup>33.</sup>  
~~112.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said second reagent comprises Tartrazine.

<sup>33.</sup>  
~~113.~~ (previously added) The system of claim <sup>26</sup>~~105~~, wherein said photon reducing agent improves the optical signal to noise ratio by at least 300 % compared to the optical signal to noise ratio in the absence of said at least one photon reducing agent.

In re Application of:  
Knapp et al.  
Application No.: 09/759,629  
Filed: January 12, 2001  
Page 7

PATENT  
Attorney Docket No.: AURO1290-4

<sup>34</sup>  
~~114~~. (previously added) The system of claim ~~80~~<sup>1</sup>, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 50 % of the concentration of said photon reducing agent outside of said plurality of living cells.

<sup>35</sup>  
~~115~~. (previously added) The system of claim ~~80~~<sup>1</sup>, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 30 % of the concentration of said photon reducing agent outside of said plurality of living cells.

<sup>36</sup>  
~~116~~. (previously added) The system of claim ~~80~~<sup>1</sup>, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 10 % of the concentration of said photon reducing agent outside of said plurality of living cells.

<sup>37</sup>  
~~117~~. (currently amended) The system of claim ~~80~~<sup>1</sup>, wherein the source of excitation light is positioned such that the living cells ~~can be~~ are excited at an excitation angle at a right angle to the aqueous reagent mixture surface.

<sup>38</sup>  
~~118~~. (currently amended) The system of claim ~~117~~<sup>37</sup>, wherein the detector is positioned such that emitted light ~~can be~~ is collected at about 12.5 degrees from the excitation angle.

<sup>39</sup>  
~~119~~. (new) The system of claim ~~80~~<sup>1</sup>, further comprising excitation light, wherein the excitation light is in contact with the reagent mixture.